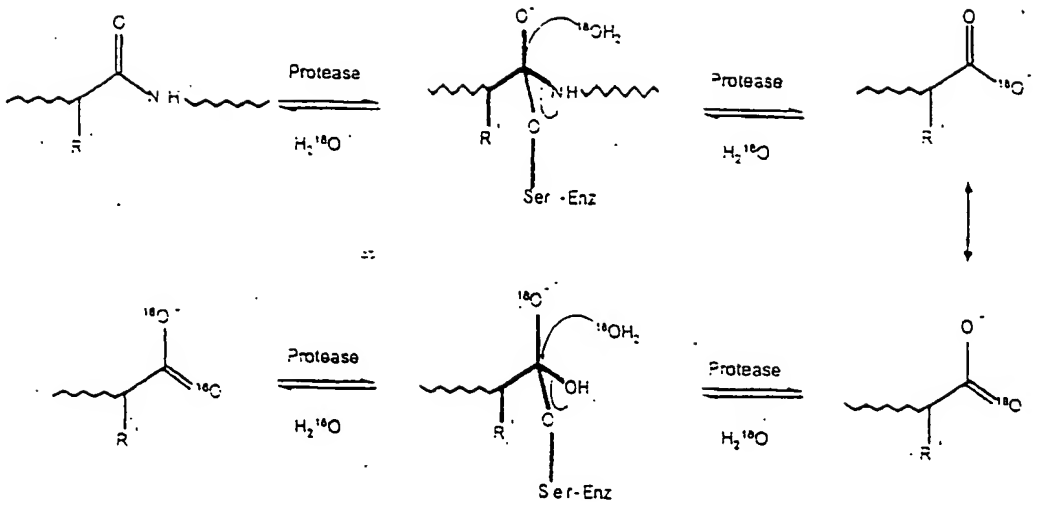
Protease
$H_2{}^{18}O$
Protease
$H_2{}^{18}O$
Ser -Enz
Protease
$H_2{}^{18}O$
Protease
$H_2{}^{18}O$
Ser-Enz

Figure 1

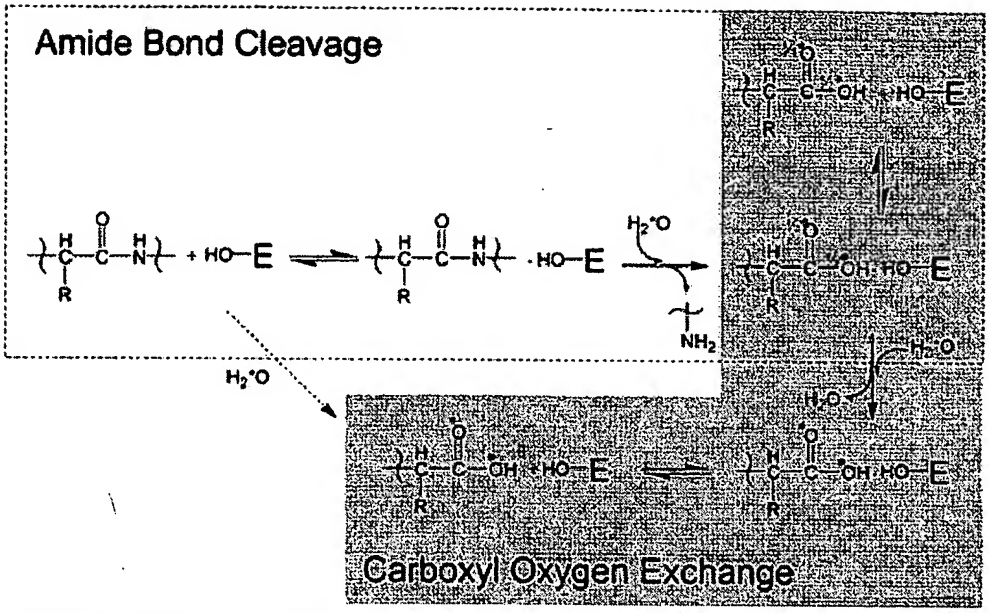

Figure 2 Dissection of Incorporation of Two Stable Isotopes during Proteolysis

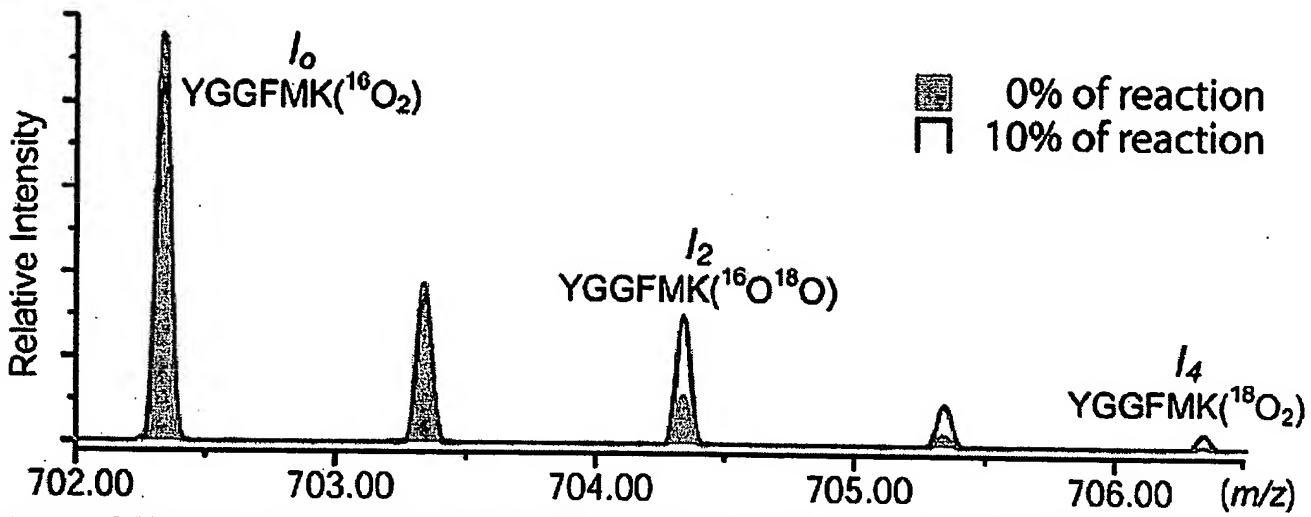

Figure 3 MALDI-FT-ICR spectra of YGGFMK at 0% and 10% conversion of the $^{16}O_2$-peptide. Two spectra were normalized according to a total concentration of $^{16}O_2$-peptide, $^{16}O^{18}O$ -peptide, and $^{18}O_2$-peptide.

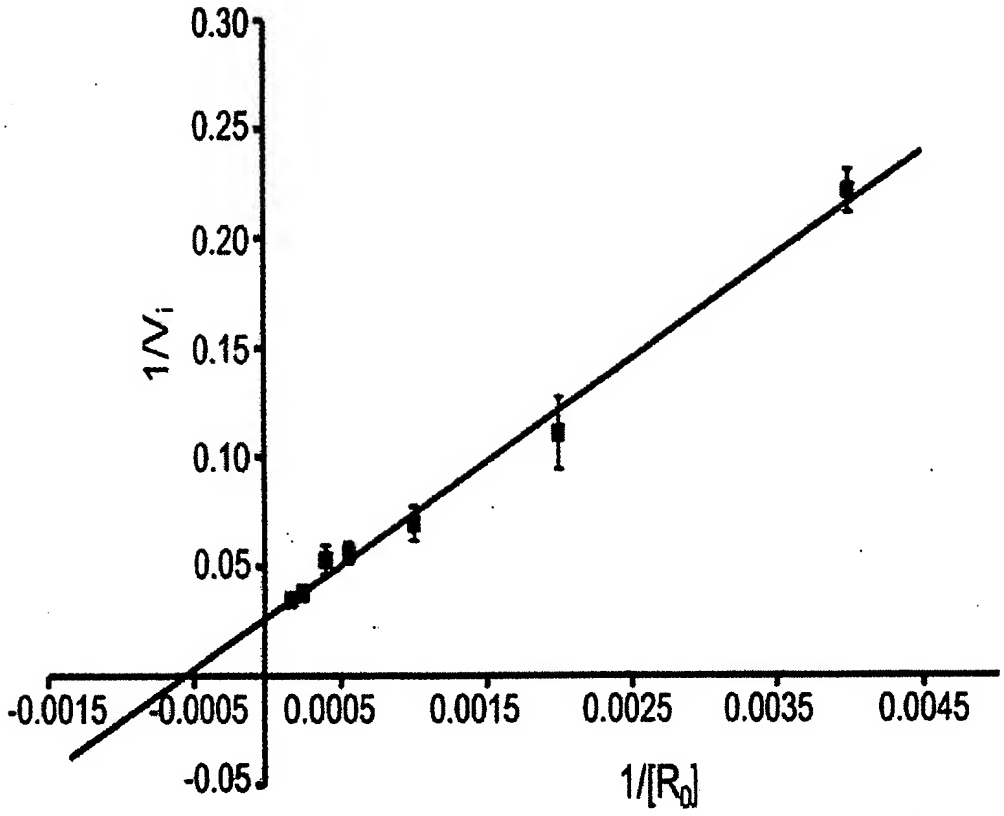

Figure 4 Double reciprocal plot of $1/v_i$ vs $1/[R_o]$ for trypsin-catalyzed ^{16}O-to-^{18}O exchange reaction of YGGFMR.

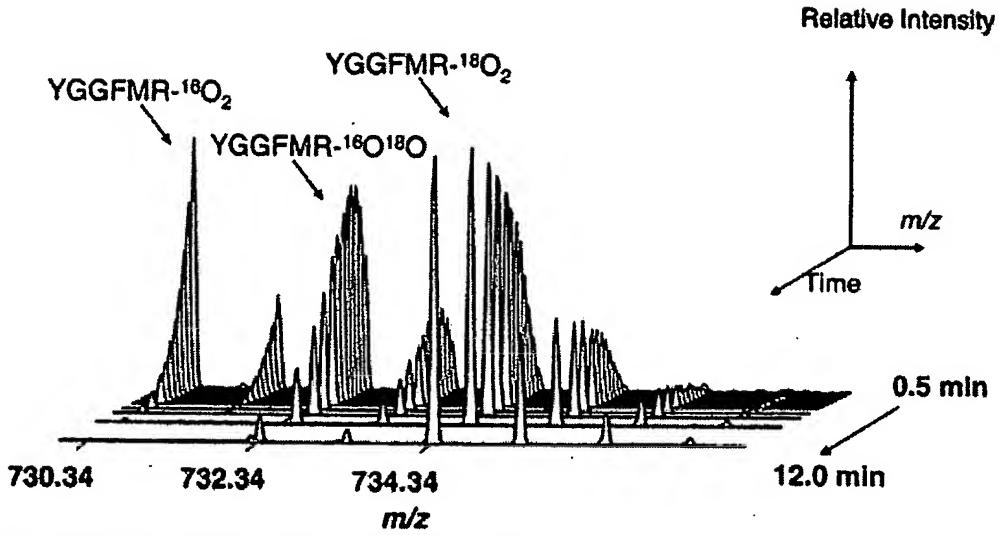

Figure 5 Sequential exchange of two carboxyl oxygens by trypsin catalysis. Inset indicates three axes.

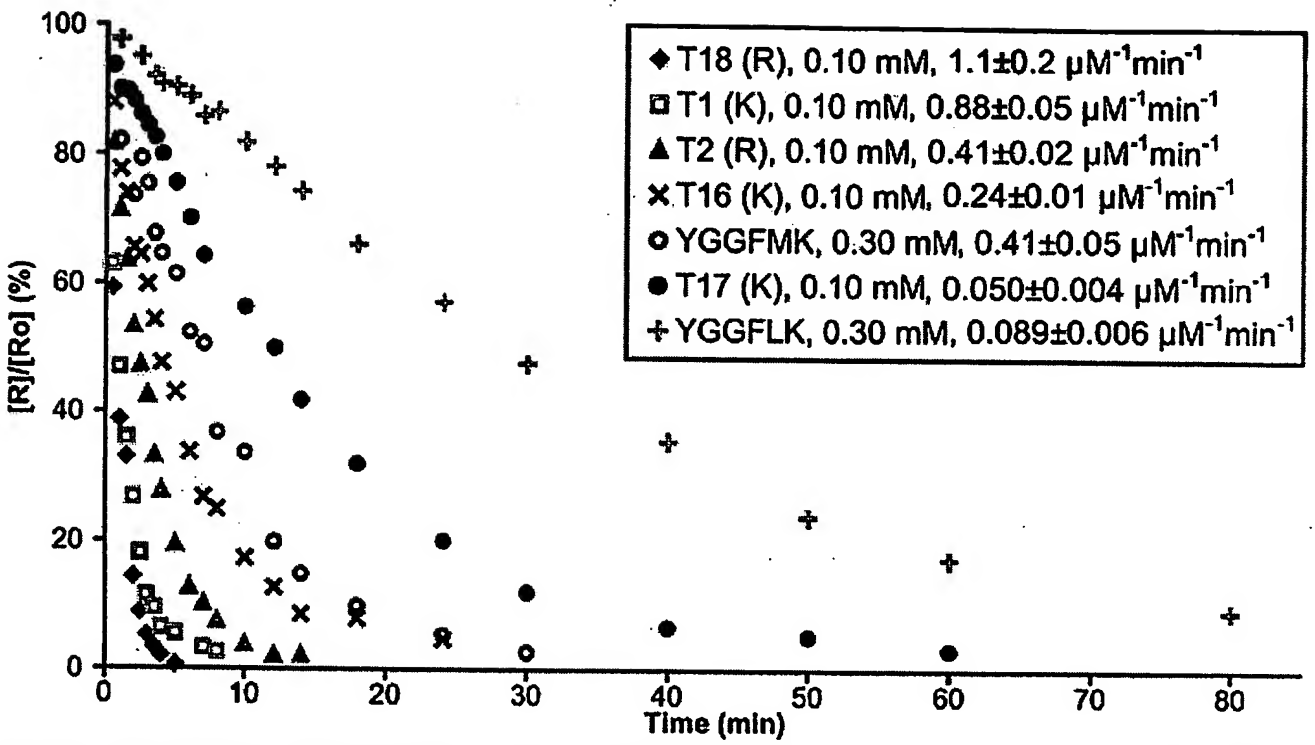

Figure 6 Simultaneous determination of pseudo first-order decay of multiple truncated peptides($^{16}O_2$). They are YGGFMK($^{16}O_2$), YGGFLK($^{16}O_2$), and five $^{16}O_2$-peptides from apomyoglobin. R's and K's in brackets represent the peptide P_1 residues. Rate constants (k_{cat}/K_M's) in the inset were calculated, using k_{cat}/K_M of YGGFMK as an internal standard. Data were based on a single experiment.

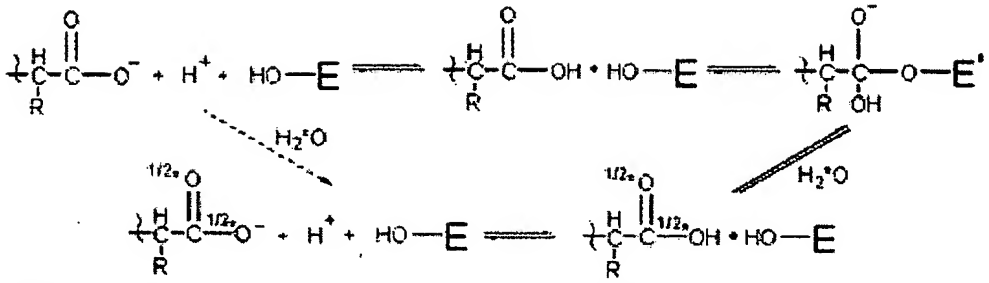

Figure 7 Sequential Exchange of Two Carboxyl Oxygens by Serine Protease: Incorporation of the First Oxygen Isotope

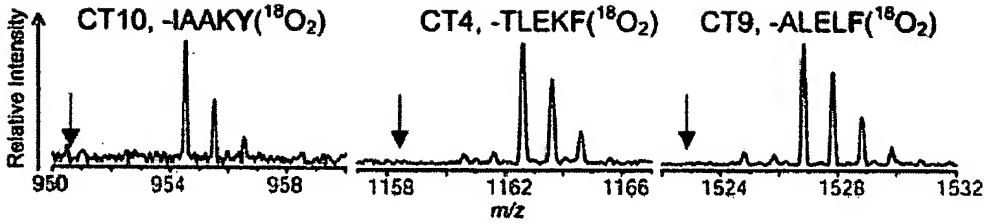

Figure 8 MALDI FT-ICR spectra of peptides $^{18}O_2$-encoded by chymotrypsin. The last five amino acid residues to the carboxyl termini for three chymotryptic peptides are shown. Arrows point to would-be positions for the peptides with ($^{16}O_2$).

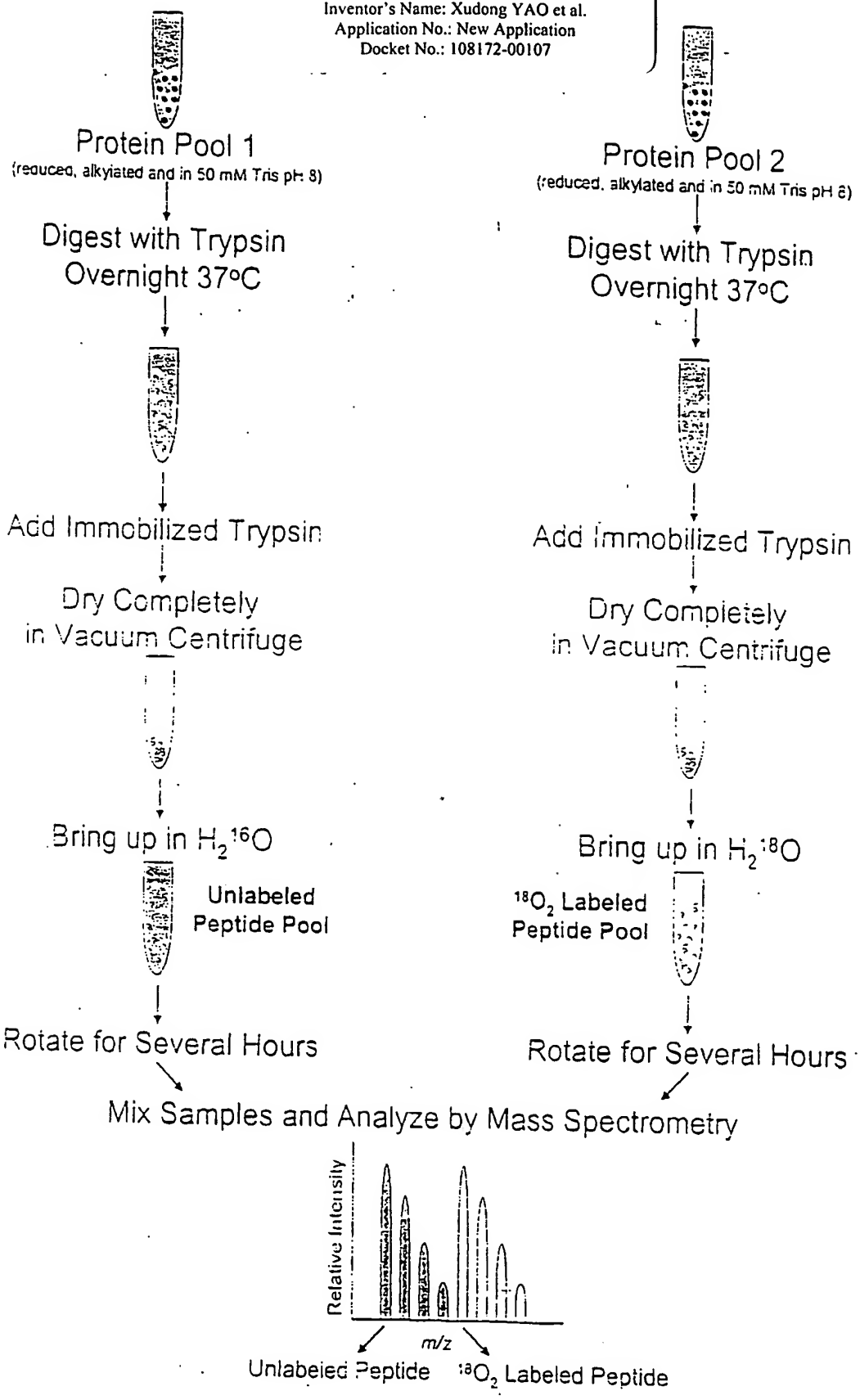

Figure 9

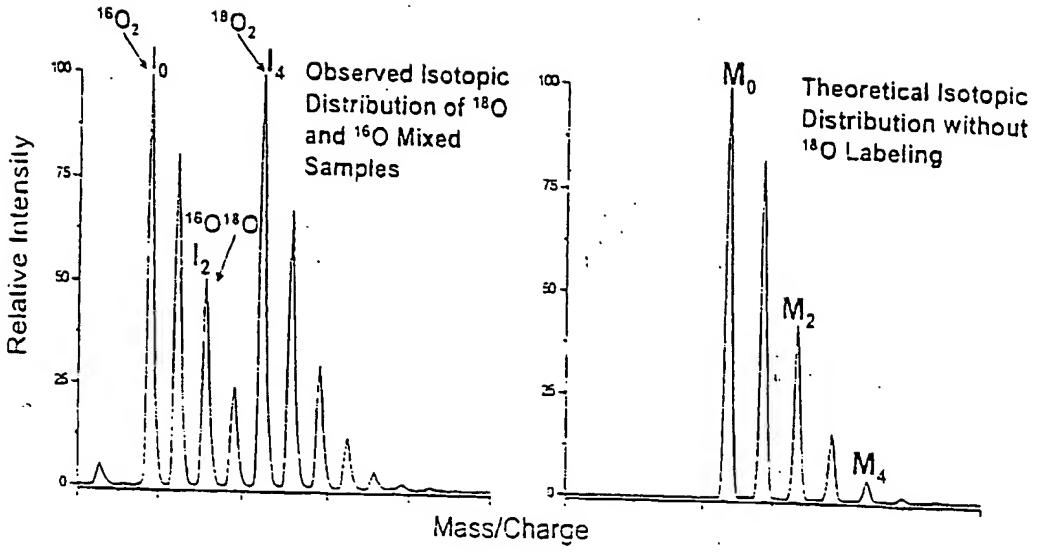
$^{16}O_2$
$^{18}O_2$
I_0
I_4
Observed Isotopic Distribution of ^{18}O and ^{16}O Mixed Samples
$^{16}O^{18}O$
I_2
Relative Intensity
100
75
50
25
0
M_0
M_2
M_4
Theoretical Isotopic Distribution without ^{18}O Labeling
Mass/Charge

Figure 10